A MENSURATIVE STUDY OF THE CYSTS OF *ENTAMOEBA COLI*

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INTRODUCTION

Several workers have recently drawn attention to the variation in size of the cysts produced by two of the common intestinal entamoebae of man—*E. histolytica* and *E. coli*. Wenyon and O'Connor (1917), in their exhaustive investigations into problems relating to amoebic dysentery, have dealt with this subject, making particular reference to the cysts of *E. histolytica*, and Dobell and Jepps (1918), after a detailed study of the cysts of this organism, have concluded that it is really a collective species comprising a number of distinct races or strains, which can be distinguished from one another by the size of their cysts. It is natural to suppose that *Entamoeba coli* is also a collective species. Wenyon and O'Connor state (1917, p. 69): 'It is very probable that strains of *E. coli* exist as we have described for *E. histolytica*, one strain differing from another in the average size of its cysts. We have not, however, made any definite measurements to decide this point.' The suggestion that at least two strains occur has been made recently by Smith (1918). Since it was considered worth while to produce further evidence bearing upon the question, the measurement of a large number of *E. coli* cysts was undertaken, and in the following pages the results of this work are briefly recorded.

MATERIAL USED AND METHODS EMPLOYED IN MAKING THE MEASUREMENTS

Cysts were measured from the stools of twenty-seven cases, who, so far as is known, became infected in different geographical regions. Of the twenty-seven cases studied, it may be mentioned that eleven (Cases 1, 2, 9, 10, 15, 18, 19, 20, 23, 26 and 27) had never been out

of England. The others were soldiers returned from various fronts. The stools from which the cysts were obtained were formed or semi-formed. In such material the great majority of *E. coli* cysts (nearly 90 per cent., as shown by Smith (1918)) are fully developed, i.e. eight-nucleate.

Cysts were measured either in saline or in Weigert's iodine solution, the latter being used if the material contained *E. histolytica* cysts as well as those of *E. coli*. It has been shown by Dobell and Jepps (1918) that iodine has no effect upon the size of the cysts of the former species, and there is no reason to suppose that cysts of *E. coli* are dissimilar in this respect. No further consideration, therefore, need be given to the question as to whether the average size of a series of measurements was obtained from cysts examined in iodine or in saline.

All measurements were made with a No. 3 ocular carrying a finely-ruled micrometer scale, in combination with a 1/12 in. oil immersion objective. The size of each cyst was recorded as the diameter in the case of spherical cysts, and as the mean of the major and minor axes in the case of elliptically-shaped cysts. The microscope was always adjusted so that one division of the micrometer scale represented 0·75 μ. It was not considered possible to estimate any measurement nearer than one division, i.e., the micrometer scale division was the actual unit of measurement employed. It is obvious, therefore, that a considerable error must enter into individual measurements, and in consequence it was decided to measure a large number of cysts from at least those infections that were being studied in detail so that the error might be as far as possible eliminated.* In two cases over one thousand cysts have been measured, but the measurement of so large a number, or even of five hundred, from more than a few cases is no inconsiderable task, and in scanty infections it becomes impossible. In some instances only one hundred cysts were measured, and at the outset it seemed desirable to ascertain what value could be attached to the average size obtained from so small a series. This point, therefore, will now be considered.

* Since the division of the micrometer scale was the unit of measurement employed, a cyst recorded as measuring 20 divisions may equally well be any value between 19·5 and 20·5 divisions, i.e. the actual size of the cyst lies between 14·65 and 15·35 μ.

VALUE OF AN AVERAGE OBTAINED FROM MEASUREMENT OF ONE HUNDRED CYSTS

For the purpose of this preliminary investigation, one thousand cysts were measured from a stool passed by Case 3, and five hundred from a stool passed by Case 22. In the former instance ten cover-slip preparations were made, and one hundred cysts measured from each, while in the latter, one hundred cysts were measured from each of five cover-slip preparations. The results are set forth in Table I.

Table I.

Case	Preparation	Number of cysts measured	Range in size in μ	Size of greatest frequency in μ	Average diameter in μ
3	1	100	12·75 – 21·0	15·0	15·44
	2	100	12·0 – 19·5	15·0	15·34
	3	100	12·0 – 20·25	15·0	15·33
	4	100	11·25 – 18·75	15·0	15·10
	5	100	12·75 – 21·0	15·0	15·45
	6	100	12·75 – 19·5	15·0	15·19
	7	100	12·75 – 19·5	15·0	15·38
	8	100	12·0 – 19·5	15·0	15·13
	9	100	11·25 – 19·5	15·0	15·31
	10	100	12·75 – 18·75	15·0	15·35
	Total	1000	11·25 – 21·0	15·0	15·30
22	1	100	15·0 – 23·25	18·75	18·53
	2	100	15·0 – 21·75	18·75	18·43
	3	100	14·25 – 22·5	18·75	18·74
	4	100	14·25 – 24·0	18·75	18·67
	5	100	15·0 – 24·0	18·0 and 18·75 equal	18·88
	Total	500	14·25 – 24·0	18·75	18·65

Inspection of Table I shows several points of interest. It seems clear that the two cases were infected with *E. coli* producing cysts

of different sizes, but this point will be dealt with later. The facts are presented in their present form to show the differences in the mean sizes obtained from the measurement of successive samples of one hundred cysts taken at random from the same stool. In Case 3 the averages vary from 15·10μ to 15·45μ, while in Case 22 they vary from 18·43μ to 18·88μ. In each instance the difference between the lowest and the highest average is approximately 0·4μ. We may conclude, then, that the mean size of cyst obtained from the measurement of one hundred is liable to an error of 0·4μ, and generally it will be less than this. For practical purposes it may be contended that this error is insignificant, but it is not altogether negligible in any detailed work relating to size-strains in minute organisms, and the advantage of having some idea of its magnitude will become evident later. The average size of the total number of cysts (one thousand) measured from one stool of Case 3 was 15·30μ. For the first five hundred the average was 15·33μ, while the second gave an average of 15·27μ. It seems, therefore, that a reliable average can be obtained from the measurement of five hundred cysts.

DETAILS OF MEASUREMENTS FROM TWENTY-SEVEN CASES

Details relating to the dimensions of cysts measured from twenty-seven different cases are given in Table II. Although the mean sizes have been obtained from the measurement of different numbers of cysts, it is convenient to arrange them in ascending order of size.

It will be seen from Table II that the average size of cyst varies in the cases studied from 15μ to 19·5μ, and that an almost regular gradation in size occurs between these two extremes. From this it might be concluded that there is little evidence for the existence of size-strains within the species. But if we consider the sizes of greatest frequency instead of average sizes, it becomes not impossible to regard the cases as falling into four fairly well-defined groups. These groups have been indicated in the Table. Group I includes Cases 1 to 8, in which the size of greatest frequency is always 15μ with a range from 11·25μ to 21·75μ. There seems little reason to doubt that these eight cases were infected with the same strain of *E. coli*—a strain producing cysts whose commonest size is 15μ, and whose average size is a little over 15μ. Case 3 is regarded as

Table II.

	No. of Case	Number of cysts measured	Range in size in μ	Size of greatest frequency in μ	Average diameter in μ
Group I	1	100	12·0 – 18·75	15·0	15·0
	2	200	12·0 – 18·75	15·0	15·0
	3	1400	11·25 – 21·0	15·0	15·3
	4	100	12·0 – 21·0	15·0	15·3
	5	100	12·75 – 19·5	15·0	15·3
	6	200	12·0 – 21·75	15·0	15·6
	7	100	12·0 – 18·75	15·0	15·7
	8	100	12·75 – 21·0	15·0	15·8
Group II	9	100	11·25 – 21·0	15·0 and 18·0	16·1
	10	100	12·75 – 21·0	15·0 „ 16·5	16·1
	11	200	12·75 – 23·25	16·5 „ 18·75	16·6
	12	100	13·5 – 21·75	16·5 „ 18·0	16·6
	13	200	12·75 – 22·5	15·75 „ 18·75	16·8
	14	100	13·5 – 26·25	15·75 „ 18·75	17·2
	15	100	13·5 – 21·75	18·0	17·6
	16	500	13·5 – 27·75	16·5 and 18·75	17·7
Group III	17	100	14·25 – 22·5	18·75	18·3
	18	100	14·25 – 24·0	18·0	18·3
	19	500	14·25 – 26·25	18·75	18·3
	20	100	15·0 – 23·25	18·75	18·4
	21	200	14·25 – 24·0	18·75	18·5
	22	1300	14·25 – 25·5	18·75	18·5
	23	100	14·25 – 23·25	18·75	18·6
	24	100	14·25 – 26·25	18·75	18·7
	25	100	15·75 – 25·5	18·75	18·7
	26	100	15·75 – 24·0	18·75	18·9
Group IV	27	500	14·25 – 27·75	18·75 and 21·75	19·5

representative of the group, and the mean size of cyst (15·3μ) may be taken as reliable since one thousand four hundred cysts were measured. The variations which occur in the average size of cyst for the remaining cases of the group are probably not significant, especially when we bear in mind the correction factor for an average obtained from only one hundred measurements.

Passing now to Group III (Cases 17 to 26), it will be found that the cysts measured from these cases range from 14·25μ to 26·25μ, the greatest frequency (with the exception of Case 18) occurring at 18·75μ. Here, again, it seems probable that the ten cases of Group III were infected with the same strain of *E. coli*—in this instance, however, a strain producing cysts whose size of greatest frequency is 18·75μ, and whose average size is somewhat below this. The average (18·5μ) obtained for one thousand three hundred cysts measured from Case 22 may be regarded as reliable, and the mean sizes obtained for the other cases in this group arrange themselves fairly closely round this value. The results so far considered, then, point to the existence of at least two strains of *E. coli*, and the evidence for this may be presented more fully by giving the actual measurements for the two cases studied in detail, viz., Cases 3 and 22. The distribution according to size of one thousand cysts from each of these cases is shown by the following figures:—

Dimensions of cysts in microns.

	11·25	12·0	12·75	13·5	14·25	15·0	15·75	16·5	17·25	18·0	18·75
Case 3	2	5	12	110	169	283	180	122	69	27	11
Case 22 ...	...	...	...	...	4	16	33	78	136	192	248

	19·5	20·25	21·0	21·75	22·5	23·25	24·0	Average size
Case 3	6	2	2	...	...	...	...	15·3μ
Case 22	123	73	48	33	10	3	3	18·5μ

These figures show clearly the differences in the dimensions of the cysts in the two strains—differences which are perhaps better shown by plotting the figures in curves (fig. 1). The two curves are of the same form, unimodal and approximately symmetrical.

There is no clear indication in either case of the cyst population having been mixed, and it is considered as established that these two curves represent two single or pure strains of *E. coli*, distinguishable as follows by the size of the cysts they produce:—

(1) Cysts of greatest frequency, 15μ; average size, 15·3μ.

(2) Cysts of greatest frequency, 18·75μ; average size, 18·5μ.

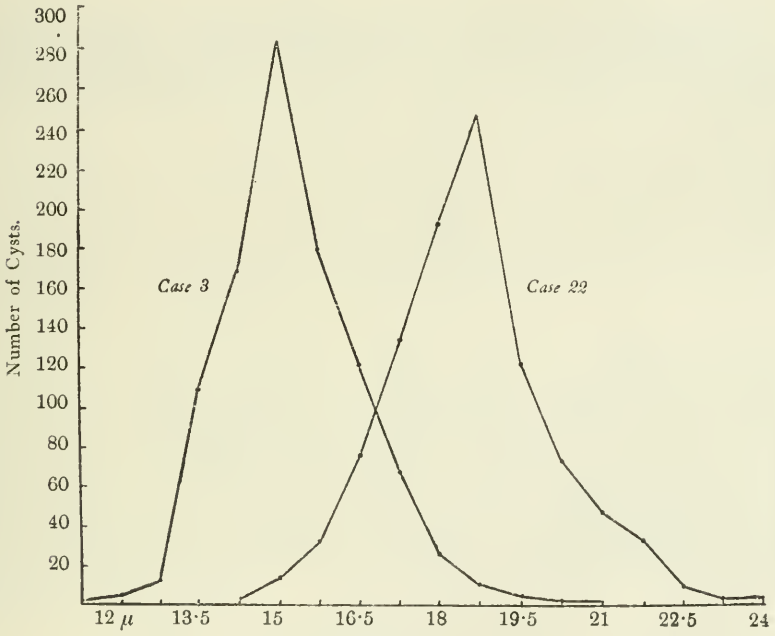

Fig. 1.

We may now consider the cases assigned to Groups II and IV of Table II. The average size of the cysts for the cases in Group II varies between 16·1μ for Case 9 and 17·7μ for Case 16. If we assume that a size-strain of *E. coli* exists between those just described, the average size of its cysts would approximate 17μ. An approximation to this has been obtained for Cases 13 and 14, where the average sizes are 16·8μ and 17·2μ respectively. The figures obtained for the measurements of the two hundred cysts from

Case 13 are as follows, and they are presented graphically in fig. 2:—

Dimensions of cysts in microns.

	12·75	13·5	14·25	15·0	15·75	16·5	17·25	18·0	18·75	19·5	20·25	21·0	21·75	22·5	Av. size
Case 13 ...	1	5	12	31	37	24	23	20	26	10	6	3	1	1	16·8μ

The feature of the curve is the presence of two apices; it is not unimodal as are the curves shown in fig. 1. The suggestion at once

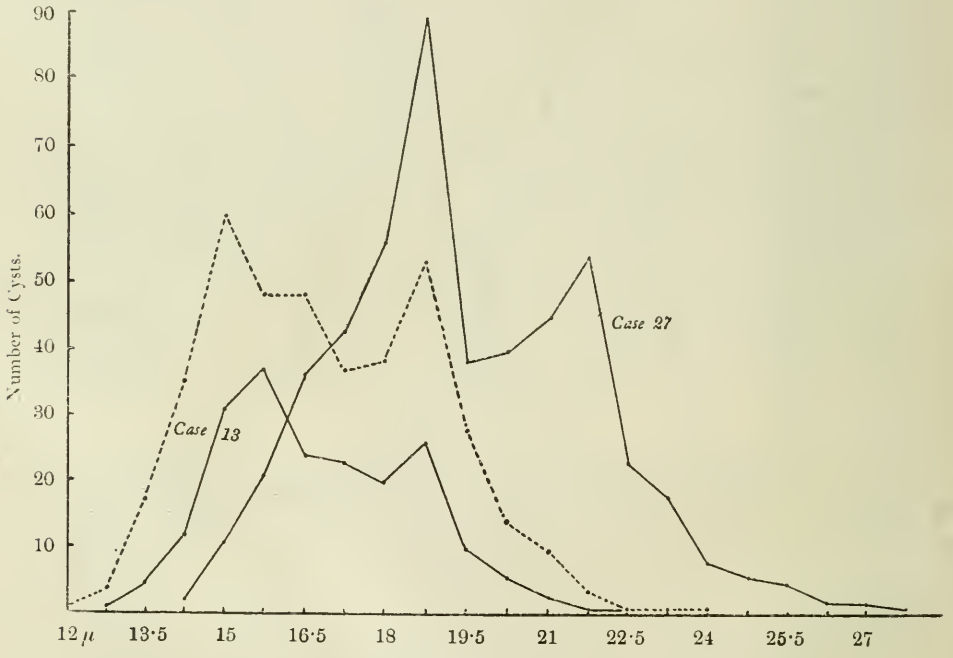

Fig. 2.

occurs that the curve for Case 13 may represent a mixed cyst population—that in this case the infection consisted of two strains co-existing in the same patient. Indeed, it seems probable that this case was infected with the two size-strains already defined. Whether this is the correct interpretation of the curve or not is very difficult to decide, and unfortunately the number of cysts measured is rather

small, but the following consideration lends some support to the idea. The dotted curve in fig. 2 represents the figures obtained by adding together the first two hundred cysts measured from Cases 3 and 22 respectively. The composition of this dotted curve is therefore known. The measurements it represents are those of two hundred cysts of the 15μ strain and two hundred of the 18·75μ strain. It will be seen that the curve so obtained is bimodal, and it is believed that the curve for Case 13 represents a similar condition, namely, a mixed cyst population. Possibly all the cases of Group II (Table II) may be explained in this way, for, with one exception, the frequency curves show two apices. It has to be noted, however, that the two apices are not at the same value in all the cases, and it is not clear that the variations may be due to different proportions of cysts belonging to the 15μ and 18·75μ strains existing in the same patient. Cases 10, 11, 12 and 16 suggest that a strain may exist producing cysts whose size of greatest frequency is 16·5μ, but in the absence of a pure infection with such a strain, its existence must for the present be left undecided.

Case 27, which is placed by itself in Group IV of Table II, must be considered separately. The first inspection of the cysts from the stool of this case at once showed that here was an infection containing many cysts obviously much larger than one generally finds in *E. coli* infections. Consequently, five hundred cysts were measured, their distribution according to size being shown by the following figures:—

Dimensions of cysts in microns.

	14·25	15·0	15·75	16·5	17·25	18·0	18·75	19·5	20·25	21·0
Case 27	2	11	21	36	43	56	89	38	40	45

	21·75	22·5	23·25	24·0	24·75	25·5	26·25	27·0	27·75
Case 27 ...	54	23	18	8	6	5	2	2	1

The curve representing these figures is shown in fig. 2. It is bimodal with one mode at 18·75μ, which seems to indicate without much doubt the presence of cysts of the strain corresponding to this size. But the curve possesses a secondary, yet distinct apex at

21·75μ, which can scarcely be explained unless on the theory that a strain of *E. coli* exists producing cysts whose size of greatest frequency is 21·75μ. Unfortunately a pure infection with cysts of this strain has not been found during this investigation, and the average size of cyst has therefore not been obtained. Judging from the second portion of the curve for Case 27, however, the average diameter would probably be a little under 21·75μ.

Bringing together the results of the present work, then, we have some evidence for believing that *E. coli* is a collective species comprising at least three strains or races which can be distinguished by the size of their cysts. The approximate dimensions of the cysts in each of these three strains are as follows:—

1. Cysts of greatest frequency, 15μ; average diameter, 15·3μ.*
2. „ „ „ 18·7μ; „ „ 18·5μ.
3. „ „ „ 21·7μ; „ „ (?) 21·5μ.

A cyst from each of these strains is shown in fig. 3, the commonest size of each having been selected for the purpose of illustrating the different races.

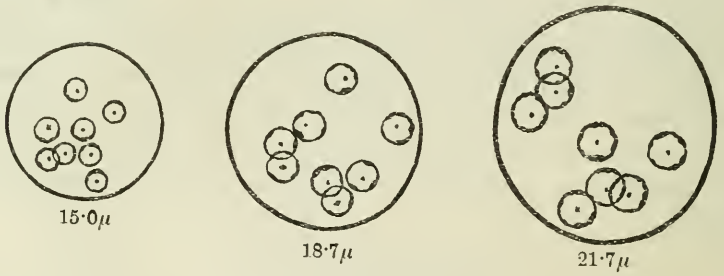

Fig. 3. A cyst of each of the three size-strains described in the text. Drawn to the same scale, under 1/12 in. oil immersion and No. 8 ocular, with the aid of a camera lucida.

While only three size-strains or races of *E. coli* have here been recognised, it is not to be concluded that this is the actual number which exists. Had it been possible to study fully a greater number of infections, it is probable that other strains would have been discovered. Wenyon and O'Connor, although they do not give any definite measurements, state 'We have frequently seen infections in which practically all the cysts were over 20 microns, while once the

* If a more accurate method of measuring had been employed, it is probable that the average size would have coincided with the size of greatest frequency. The mean size obtained (15·3μ) actually lies in the same modal group of measurements (14·65–15·35μ) as the size of greatest frequency.

average size was well over 25.' This statement obviously refers to strains producing large cysts, and the first mentioned might correspond to the 21·7 μ strain which I have described. The strain which produces cysts averaging about 25 μ must be exceedingly rare.

But whatever the actual number of races, it is obviously important for the final proof of their existence to show that they remain constant in size. To do this it is necessary to measure cysts from the same case at intervals over a considerable period of time, and in this connection it is useful to know what value may be attached to the mean size obtained from the measurement of, say, one hundred cysts. It is not always possible to measure large numbers. We have already seen that the average size of one hundred measurements is liable to an error of approximately 0·4 μ. With this in mind, we may consider the results presented in Table III, which shows the average size of cysts obtained from the measurement of various samples from Cases 3 and 22 on different dates.

Table III.

Case	Date of examination	Number of cysts measured	Size of greatest frequency in μ	Average diameter of cyst in μ
3	19.3.18	100	15·0	15·32
	23.3.18	100	15·0	15·17
	26.3.18	100	15·0	14·98
	5.4.18	1000	15·0	15·30
	24.4.18	100	15·0	15·02
22	27.5.18	500	18·75	18·40
	15.6.18	500	18·75	18·65
	18.6.18	100	18·75	18·65
	9.7.18	100	18·75	18·74
	12.7.18	100	18·75	18·41

For Case 3 no significant change in size occurred during the five weeks when observations were made. For Case 22 when, on two occasions, five hundred cysts were measured at an interval of nearly three weeks, the difference of 0·25 μ in the mean size is perhaps a

little greater than might have been expected from so large a sample. In the interval between the two observations the patient had received treatment with emetine bismuth iodide for a concurrent infection of *E. histolytica*. It is not impossible that the slight increase in size of the *E. coli* cysts which re-appeared after treatment is in some way connected with the administration of the drug. Dobell and Jepps have drawn attention to the larger size of cysts of *E. histolytica* which first appear in a relapse after treatment, and Smith has recorded a similar phenomenon for *E. coli* cysts. It appears, therefore, from the results set forth in Table III that, if any change in mean size of cyst does occur from day to day, it is comparatively small, and there seems little doubt that if a case be infected with a *pure* strain producing cysts averaging about 15μ then that case will continue to pass cysts having that average size. Similarly, an infection with a *pure* strain producing cysts about 18·5μ will remain constant. We should not expect, for instance, that the one would suddenly change into the other. It is obvious, of course, that if a case be infected with two strains the number of cysts passed from day to day belonging to the different strains may vary, and there may be consequently a considerable daily variation in the mean size of cyst. As already pointed out, it is considered possible to regard all the cases comprising Group II of Table II as having double infections, i.e. they were infected with two size-strains of *E. coli*. Unfortunately, only in two cases were cysts measured on different days. The first hundred cysts measured from Case 11 averaged 16·4μ, while from a stool passed three days later the average of one hundred was 16·9μ. In the second instance, Case 13, the average obtained from the first measurement of one hundred cysts was 16·5μ, and a fortnight later a second series of one hundred gave 17·1μ as the average diameter. These differences are not outstanding, although they exceed the error likely to be obtained through using a small sample, and it is suggested that they may lend further support to the theory already advanced that each of the Cases 9 to 16 had a double infection, and constancy in the mean size of cysts passed from day to day would not therefore be likely to occur.

Frequency curves for the size of *E. coli* cysts have been given by various workers. Kuenen and Swellengrebel (1913) give a curve which shows two apices—one at 18μ and another at 20μ—with a dip at 19μ. Since the curve is based on only one hundred cysts, it is

difficult to draw any conclusions from it, but if smoothed out, it might be interpreted as representing a strain corresponding fairly closely to the 18·7μ strain which I have described.

A frequency curve given by Mathis and Mercier (1917) shows three well-defined apices corresponding to cysts measuring (in the fresh state) 16·5μ, 18μ and 19·5μ. The remarkable feature of their curve, however, is the fact that only three or four cysts exist between these sizes—a feature which I cannot understand since the apices in the curve fall so closely together. Even if it be admitted that strains occur of the sizes given by these authors, it would be necessary, in order to explain their curve, to assume that each strain showed practically no range in the size of its cysts. Unfortunately, Mathis and Mercier are satisfied with a curve which represents one hundred measurements, and it is difficult to estimate how far their results would have been modified if they had measured a much larger number of cysts.

The general curve for one thousand cysts measured from a large number of cases by Smith (1918) shows a single apex at 16·7μ, while the average size is 17·3μ. But the results shown in Table VIII of his paper suggest that his infections '9,' '10' and '11' correspond to the 18·7μ strain, while his infections '1' and '2' are probably the same as the 15μ strain. A general curve for two thousand seven hundred cysts measured during the present work is shown in fig. 4. This number has been obtained by adding together the first hundred cysts measured from each of the twenty-seven cases studied, so the contribution of each case to the total number is equal. The curve represents the following figures:—

Dimensions of cysts in microns.

11·25	12·0	12·75	13·5	14·25	15·0	15·75	16·5	17·25	18·0	18·75	19·5
1	8	24	90	183	382	305	301	287	339	353	170

20·25	21·0	21·75	22·5	23·25	24·0	24·75	25·5	26·25	27·0	27·75
90	82	36	18	12	6	2	3	5	2	1

The curve is bimodal, with modes at 15μ and 18·75μ, corresponding to the two races producing cysts of these respective sizes. The one hundred cysts contributed by Case 27 are insufficient to indicate in the general curve the 21·75μ strain found in that case.

The average diameter of the two thousand seven hundred cysts is 17·1 μ, which is almost identical with that obtained by Smith for one thousand cysts. His results and mine are, therefore, not

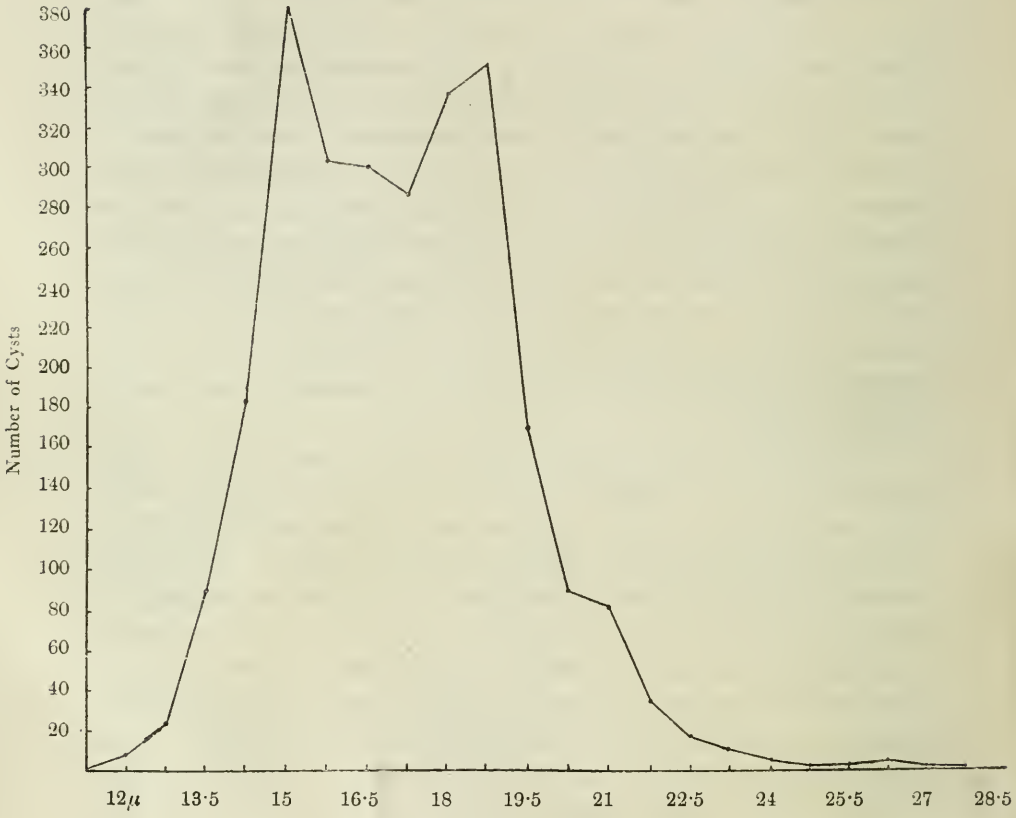

Fig. 4.

incompatible, and a general curve for the size of *E. coli* cysts may vary to some extent according to the number of cases studied, the number of strains encountered, and the number of cysts contributed by each case.

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